REMARKS

Claims 58-102 are pending in the instant application. Reconsideration and rejection of the outstanding rejections is respectfully requested. Applicants note with appreciation that the rejection of claims 58-102 under 35 U.S.C. §112, second paragraph, set forth in the Office Action mailed August 14, 2002, has been withdrawn.

Examiner Interview Summary

The Examiner (Misook Yu) and the Supervisory Examiner (Anthony Caputa) are kindly thanked for meeting with Applicant Dr. Michael S. Kinch and Applicants' Representatives Jonathan Klein-Evans, Edward Amaya and Applicant's Representative Victoria Sandberg (the latter by telephone) to conduct a personal interview in this matter. All claims were discussed.

It was agreed that the Applicants would supply *in vivo* data to address the rejection under 35 U.S.C. §112, first paragraph. Also, the participants reviewed Koolpe et al. (J. Biol. Chem. 277:49, 46974-46979, 2002) as an example of how one of skill in the art can use Applicants' teachings to identify compounds, such as peptides, that increase the phosphotyrosine content of, and thereby activate, the EphA2 receptor. Applicants also clarified the record to indicate that it was discovered that antibody B2D6 exerts an agonistic (tyrosine autophosphorylation) effect on EphA2 when aggregated (cross-linked) but, in contrast to the agonizing antibodies EA2 and B233 reported in the Declaration of Dr. Michael S. Kinch submitted herewith, does not have an appreciable stimulating effect when not aggregated.

Information Disclosure Statements Mailed February 14, 2001, March 13, 2001, September 11, 2001 and December 27, 2001

Applicants note that an earlier Office Action Summary dated August 14, 2002, indicated that three IDS documents (paper nos. 6, 10 and 12) were attached, but they were not attached and consequently were not received by Applicants' Representatives with the copy of the office action. We assume these represented IDS's filed on March 13, 2001, September 11, 2001 and December 27, 2001. The Examiner is kindly requested to provide a copy of the initialed 1449s with the next official communication.

Objection to the Specification

The specification remains objected to because the Examiner states that it is not clear that the EphrinA1-Fc used in the instant specification is commercially available. This objection is respectfully traversed.

EphrinA1, also known as B61 and LERK-1 (see McBride et al., Mech. Dev. 77(2):201-204 (1998) (abstract only); NCBI Locus Link query for B61 (performed and dated November 24, 2003); and Easty et al., Cancer Res. 55:2528-2532 (1995) all of which are submitted herewith and listed on the enclosed 1449 forms), is a well-known natural ligand for EphA2 receptor (previously called Eck receptor). The chimera EphrinA1-Fc (also known as B61-Ig) is also well known to the art, and its recombinant synthesis is described in detail in Pandey et al., Science, 268:567-569 (1995), listed on the enclosed 1449 form and submitted herewith. At the time of the invention EphrinA1-Fc could be readily made using recombinant DNA technology following the procedure described in Pandey et al. Ephrin-A1-Fc is also currently commercially available from a number of vendors, including R & D Systems (Minneapolis, MN) under the catalog number 602-A1; Sigma-Aldrich Chemical Company under the product number E9902; and Genzyme Techne (G-T) under the product number 3602. Catalog pages for the R&D Systems product and the G-T product are listed on Form 1449 submitted herewith in connection with a

Supplemental Information Disclosure Statement, and copies of the catalog pages are also provided.

Since EphrinA1-Fc was readily obtainable through standard methods at the time of filing of the instant application, Applicants submit that the specification sufficiently describes EphrinA1-Fc. Reconsideration and withdrawal of the objection to the specification is accordingly requested. If the Examiner continues to object to the specification, further clarification of the basis of the objection, and specific suggestions for overcoming it, are kindly requested.

Rejection under 35 U.S.C. §112, First Paragraph

Claims 58-102 remain rejected under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. The Examiner alleges that, at the time the instant application was filed, the Applicants had possession of only one compound, EphrinA1-Fc, and only one antibody, B2D6, that increase the phosphotyrosine content of EphA2, and that based on those compounds one cannot predict the types of additional compounds that might increase phosphotyrosine content of the EphA2 receptor. Applicant respectfully traverses the rejection.

The invention is directed to the use a compound that increases the phosphotyrosine content of EphA2 to treat metastatic cancer. Applicants' invention is based on their elucidation of the relationship between EphA2 and cancer, and more particularly on their surprising discovery that *activating* EphA2 in cancer cells (by, for example, causing an *increase in the phosphotyrosine content* of EphA2) has therapeutic benefit. As discussed during the personal interview, there is no teaching in the prior art to suggest this surprising and unconventional result that *agonizing* this receptor tyrosine kinase would inhibit tumor growth. Regarding the Examiner's assertion that the specification includes, as working examples, only two molecules that increase the phosphotyrosine content of EphA2, Applicants emphasize that they, indeed, had

possession of the invention commensurate with the full scope of the claims at the time of filing. One of skill in the art, using the teachings of the specification, could (and already has, as will be discussed below) readily screen and identify a wide variety of additional compounds capable of increasing the phosphotyrosine content of EphA2. Specifically, Applicants have clearly taught the following additional aspects of the invention, among others:

(1) an assay method for detecting an increase in the phosphotyrosine content of EphA2;

(2) a connection between increase in phosphotyrosine content of EphA2 and a therapeutic effect;

(3) working examples of compounds shown to increase the phosphotyrosine content of EphA2; and

(4) other types of compounds that can be readily screened according to the methods taught in the specification for their effectiveness in increasing the phosphotyrosine content of EphA2.

The assay method for phosphotyrosine content of EphA2 utilizes Western blot technology and is set forth in the specification at page 16, lines 8-14:

> To measure EphA2 stimulation, the phosphotyrosine content of immunoprecipitated EphA2 was measured by Western blot analysis with phosphotyrosine specific antibodies. Whereas the EphA2 in vector-transfected MCF-10 cells was tyrosine phosphorylated, EphA2 was not tyrosine phosphorylated in MCF^{EphA2} cells. The decreased phosphotyrosine content was confirmed using multiple EphA2 antibodies for immunoprecipitation (D7, B2D6) and different phosphotyrosine-specific antibodies (4G10, PY20) for Western blot analyses.

The types of compounds that can be readily assayed for their effect on the phosphotyrosine content of EphA2, are taught at page 3, lines 10-12 of the specification, wherein it is stated that "EphA2 may be targeted by use of artificial or hybrid forms of the protein, protein inhibitors, antisense oligonucleotides, or small molecule inhibitors."

The present invention was *reduced to practice* as shown in a working example wherein EphrinA1-Fc increased the phosphotyrosine content of EphA2 in MCF^{EphA2} cells suspended in

soft agar (specification at page 18, lines 4-16). Treatment with EphrinA1-F_c reduced colony formation in soft agar by 49% relative to vehicle-treated controls, and restored a spherical phenotype in these cells in Matrigel that was comparable to non-transformed MCF-10A cells.

Likewise the specification demonstrates in a working example a therapeutic effect using an antibody that binds EphA2, namely B2D6. Treatment of metastatic breast cancer cells with B2D6 antibody, when aggregated (cross-linked) as noted by Dr. Kinch in the personal interview, restores tyrosine phosphorylation of EphA2 in these cells and blocks about 50% of growth (specification at page 11, lines 5-10).

In elucidating the heretofore unrecognized relationship between activation of the EphA2 receptor and a therapeutic effect, and in providing two independent working examples (one involving an antibody, the other a peptide-ligand conjugate), Applicants have provided a *clear and direct path* for identification other compounds that exhibit this activity. Identification of such compounds requires at most only routine experimentation in light of the detailed teachings of the specification. The skill in the relevant art is very high, and the identification of particular compounds and methods for increasing the phosphotyrosine content of EphA2 are well within the skill of the relevant art workers, particularly in view of the assays provided in the specification.

Indeed, the methods set forth in the present application have been successfully followed to identify other compounds that agonize EphA2. For example, in Koolpe et al. (J. Biol. Chem. 277:49, 46974-46979, 2002) the Pasquale laboratory "exploited" EphA2 as a therapeutic target (thereby taking advantage of the Applicants' contribution to the art) by using phage display to identify two peptides that bound to EphA2, one of which stimulated EphA2 tyrosine phosphorylation and signaling. Phage display is a common technique to identify peptides that exhibit selectivity for their targets, and the peptide library used by Koolpe et al. was obtained from a commercial supplier (Koolpe et al., Material and Methods, at 46974, right column). Fig. 2B of Koolpe et al. (including the legend and the text relating thereto) describes probing of immunoprecipitated EphA2 with anti-physphotyrosine (PTyr) or anti-EphA2 antibodies (as

described in the present specification at page 16, lines 8-14) to detect stimulation of tyrosine phosphorylation of the receptor. It is evident that the scientific work of the Applicants provided the foundation for this discovery as publications from Dr. Kinch's laboratory were cited as references 12, 13 and 18.

In addition, Applicants' own work since the filing of the application shows that additional compounds (namely, antibodies EA2 and B233) can be identified using the methods taught in the specification (see Declaration of Michael S. Kinch under 37 C.F.R. § 1.132, submitted herewith).

In summary, Applicants were in full possession of the invention as claimed at the time of filing the application, having taught everything necessary to identify active compounds and perform the claimed method. It is not a difficult matter, in view of the Applicants' teachings, for a skilled worker to quickly identify or develop many varied compounds that increase the phosphotyrosine content of EphA2 in cancer cells. To limit the Applicant to a particular exemplary compound that increases the phosphotyrosine content of EphA2, or to a particular exemplary method of increasing phosphotyrosine content, would unfairly deprive Applicants of the protection to which they are entitled as a result of their important discovery, allowing others to reap an unjust benefit. The claimed process involves increasing the phosphotyrosine content of EphA2 in the cancer cell. Having clearly identified an assay for measuring an increase in phosphotyrosine content of EphA2, the Applicants have established possession of the claimed method as required by 35 U.S.C. §112, first paragraph.

Claims 58-102 remain rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which pertains, or with which it is most nearly connected, to make and/or use the invention. The Examiner invited Applicant to submit *in vivo* data to obviate this rejection.

Claims 58-102 also remain rejected under 35 U.S.C. §112, first paragraph, because the specification, while being enabling for B2D6 for decreasing the growth of metastatic breast cancer cells in culture and EphrinA1-Fc for reducing colony formation of EphA2-transformed

MCF cells in soft agar, does not reasonably provide enablement for any other compounds for the purpose stated in the preambles. In other words, the enablement is not commensurate in scope with the claims.

These rejections are respectfully traversed. As discussed in the interview, Applicants will address both of these enablement rejections together.

As already noted, the specification clearly sets forth a connection between phosphotyrosine content and EphA2 activity; a method for evaluating changes in phosphotyrosine content of EphA2 in a cancer cell; a wide variety of examples of agents (candidate agonists) that can be readily assayed for their effect on phosphotyrosine activity; and the use of agonists in a therapeutic context to alter the expression of EphA2 (e.g., specification at page 3, lines 9-10).

The Examiner's main concern appears to be the lack of *in vivo* data, which Applicants can now provide. Proposed *in vivo* experiments were described in detail (see e.g., the specification at page 12, line 31, through page 14, line 14; see also page 16, line 19 through page 17, line 33), along with expected results. To supplement this disclosure, Applicants submit herewith the Declaration of Dr. Michael S. Kinch under 37 C.F.R. § 1.132 detailing *in vivo* results using various compounds to treat various metastatic cancers. As detailed in the Declaration, Dr. Kinch has used two *in vitro* assays described in the present specification, namely the tyrosine phosphorylation and soft agar colony formation assays, to identify two agonistic anti-EphA2 antibodies. Furthermore, Dr. Kinch has demonstrated that the *in vitro* activities of these agonistic antibodies directly correlate with *in vivo* decrease of tumor growth. Moreover, the decrease in tumor growth was demonstrated using two different human breast cancer cell lines and a human lung cancer cell line, thereby showing the invention is applicable to a variety of tumors.

In addition, Applicants draw the Examiner's attention to the experiment reported in Example 8 at page 18. Although this is an "*in vitro*" experiment (cell culture), Matrigel is a three-dimensional culture system that much more closely approximates the natural environment

of cells than two-dimensional cultures. The correlation between cell behavior in Matrigel and cell behavior in their natural environment is therefore thought to be superior to other *in vitro* systems.

The *in vivo* experiments reported in the Declaration of Dr. Michael Kinch unequivocally support the teachings of the specification. Applicants believe the pending claims are fully enabled, commensurate with their scope.

Reconsideration and withdrawal of the rejection of claims 58-102 under 35 U.S.C. §112, first paragraph, is respectfully requested.

Summary

It is respectfully submitted that the pending claims 58-102 are in condition for allowance and notification to that effect is respectfully requested.

If it is found that the claims are not in condition for allowance, Applicants request a second non-final Office Action, in order be assured of a full and fair opportunity to respond to the complete action on the merits as described in detail above.

The Examiner is invited to contact Applicants' Representatives, at the below-listed telephone number, if it is believed that prosecution of this application may be assisted thereby.

Respectfully submitted for
Michael S. Kinch et al.

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CERTIFICATE UNDER 37 CFR §1.10:
"Express Mail" mailing label number: EV073737370US Date of Deposit: 24 November 2003
The undersigned hereby certifies that this paper is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR §1.10 on the date indicated above and is addressed to the MAIL STOP RCE, Assistant Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450
By: Jacquelyn K. Torborg
Name: Jacquelyn K. Torborg

PATENT
Docket No. 290.0010 0101

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	Michael S. Kinch et al.	Group Art Unit:	3254
Serial No.:	09/640,935	Examiner:	Misook Yu
Confirmation No.:	3254		
Filed:	17 August 2000		

For: EPHA2 AS A THERAPEUTIC TARGET FOR METASTATIC CANCER (As Amended)

DECLARATION OF MICHAEL S. KINCH, PH.D. UNDER 37 C.F.R. § 1.132

Assistant Commissioner for Patents
Washington D.C. 20231

Dear Sir:

I, Michael S. Kinch, hereby state and declare as follows:

1. I am a co-inventor of the patent application referenced above. I am currently employed as Director of Oncology at MedImmune, Inc. I received my Ph.D. in Immunology in 1993 from Duke University. I was a post-doctoral fellow at The University of North Carolina at Chapel Hill in Cancer Cell Biology from 1993-1996. I was Assistant Professor of Pharmacology at Purdue University from 1996-2001. I joined MedImmune in 2001.

2. The following experiments were carried out under my direct supervision:

3. EphA2 was immunoprecipitated from human MDA-MB-231 breast cancer cells that had been incubated with agonistic monoclonal antibodies EA2 or B233 for up to 60 minutes. The immunoprecipitated EphA2 was then resolved by SDS-PAGE and subjected to Western blot analysis with anti-P-Tyr antibodies (to detect tyrosine phosphorylated EphA2). The membranes were then stripped and re-probed with anti-EphA2 antibodies (as a control to confirm equal sample loading). These data are illustrated on exhibit A1. These data demonstrate that antibodies EA2 and B233 induce autophosphorylation of EphA2 (*i.e.* agonize EphA2).

4. Whole cell lysates from MDA-MB-231 cells that had been incubated with anti-EphA2 antibodies EA2 or B233 for up to 72 hours were blotted. Blots were probed with EA2 and B233 and a control antibody (anti-b-catenin) to confirm equal sample loading. These data are illustrated on exhibit A2. These data show degradation of EphA2 within 2 hours for B233 (complete) and some degradation for EA2 (complete at 12 hours). Therefore, these data demonstrate that EA2 and B233 induce degradation of EphA2 (*i.e.*, agonize EphA2).

5. MDA-MD-231 cells were suspended in soft agar. Growth in soft agar is an *in vitro* model for metastatic cell behavior. Antibodies EA2, B233 and a control were administered at the time of suspension. Colony formation (defined as colonies that contain at least 3 viable cells) was scored after 3 or 7 days. These data are illustrated on exhibit B. These data show that both EA2 and B233 decreased colony growth in soft agar. Therefore, these data demonstrate that EA2 and B233 decrease colony formation of cancerous cells and growth in soft agar assays.

6. Tumors from the human cancer cell lines MDA-MB-231, a breast cancer cell line, and A549, a lung cancer cell line, were implanted into mice and the tumors were allowed to become established and grow to ~200 mm^3. The mice were then treated with EA2 or a control antibody, administered twice weekly, and tumor volume was evaluated over time. The results are depicted on exhibit C. The results show that there was a reduction in tumor growth rates in mice that were treated with EA2 relative to tumors treated with the control. Thus, these data demonstrate that EA2 decreases the growth of tumors *in vivo*.

7. In a similar experiment to that shown in exhibit C, mice were implanted with MCF-7 ^{EphA2}, a human breast tumor cell line that had been transfected with human EphA2. The tumors were allowed to grow and the mice were treated with B233 or control antibody. The results are summarized on exhibit D. The results show that the tumor volume was reduced when the animals were treated with B233 as compared to control. Thus, these data demonstrate that B233 inhibits growth of tumors *in vivo*.

8. The MDA-MB-231 tumors from the experiment described in paragraph 6 above (exhibit C) were excised from the mice, extracted with detergent, and the resulting lysates were blotted (tumors from animals treated with EA2 or control). A western blot for EphA2 was performed using the EA2 antibody. These data are illustrated on exhibit E. These data indicate that EphA2 was detected in tumors from animals treated with control antibody, while EphA2 levels were

reduced in the tumors isolated from animals treated with EA2. These data show that EphA2 positive tumor cells have been eliminated from the cell population.

9. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine, or imprisonment, or both, under section 1001 of Title 18 of the Unites States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

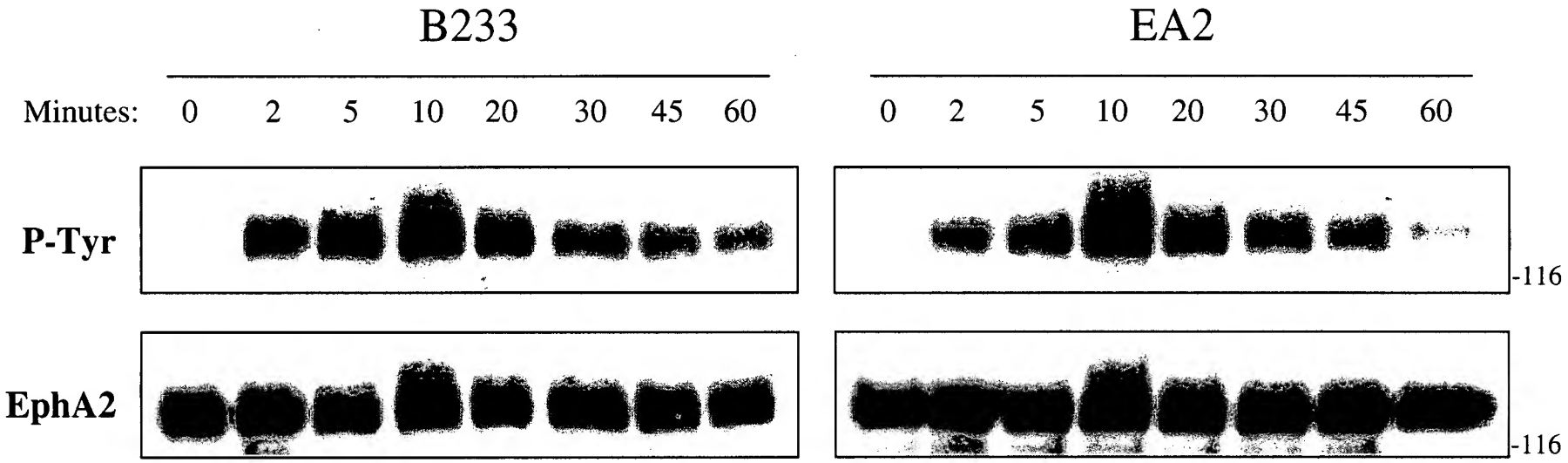
11-24-03

Date

Michael S. Kinch, Ph.D.

Exhibit A

1.



2.

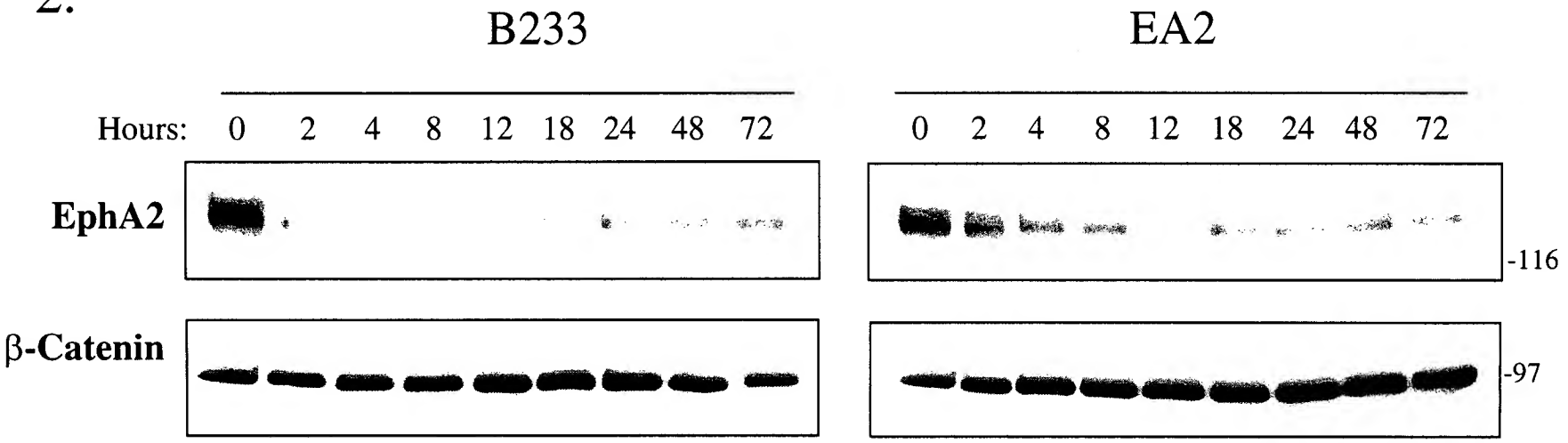


Exhibit B

Both EA2 and B233 decrease malignant (anchorage-independent) growth

MDA-MB-231

Experiment: Treatment of MDA-MB-231 cells with a single dose (5 ug/mL) of antibody, followed by visual assessment of colony formation at the indicated time.

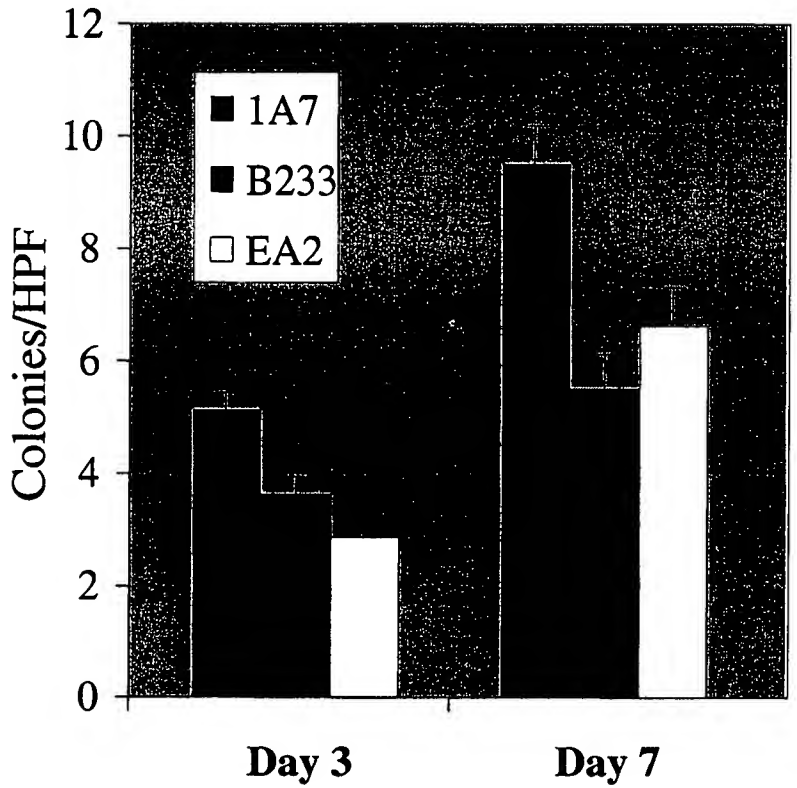


Exhibit C

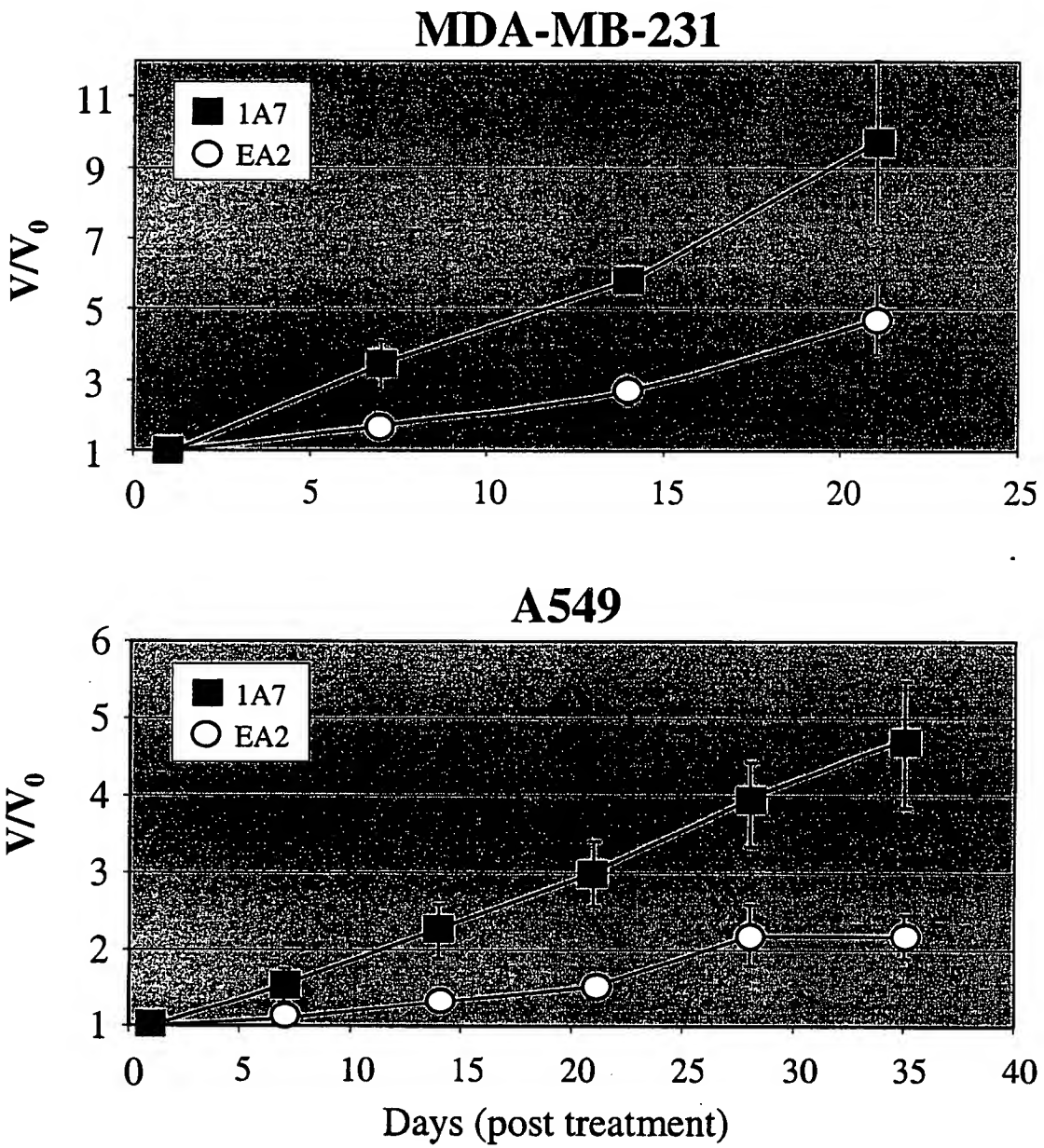


Exhibit D

B233 – Established MCF-7^{EphA2} Tumors

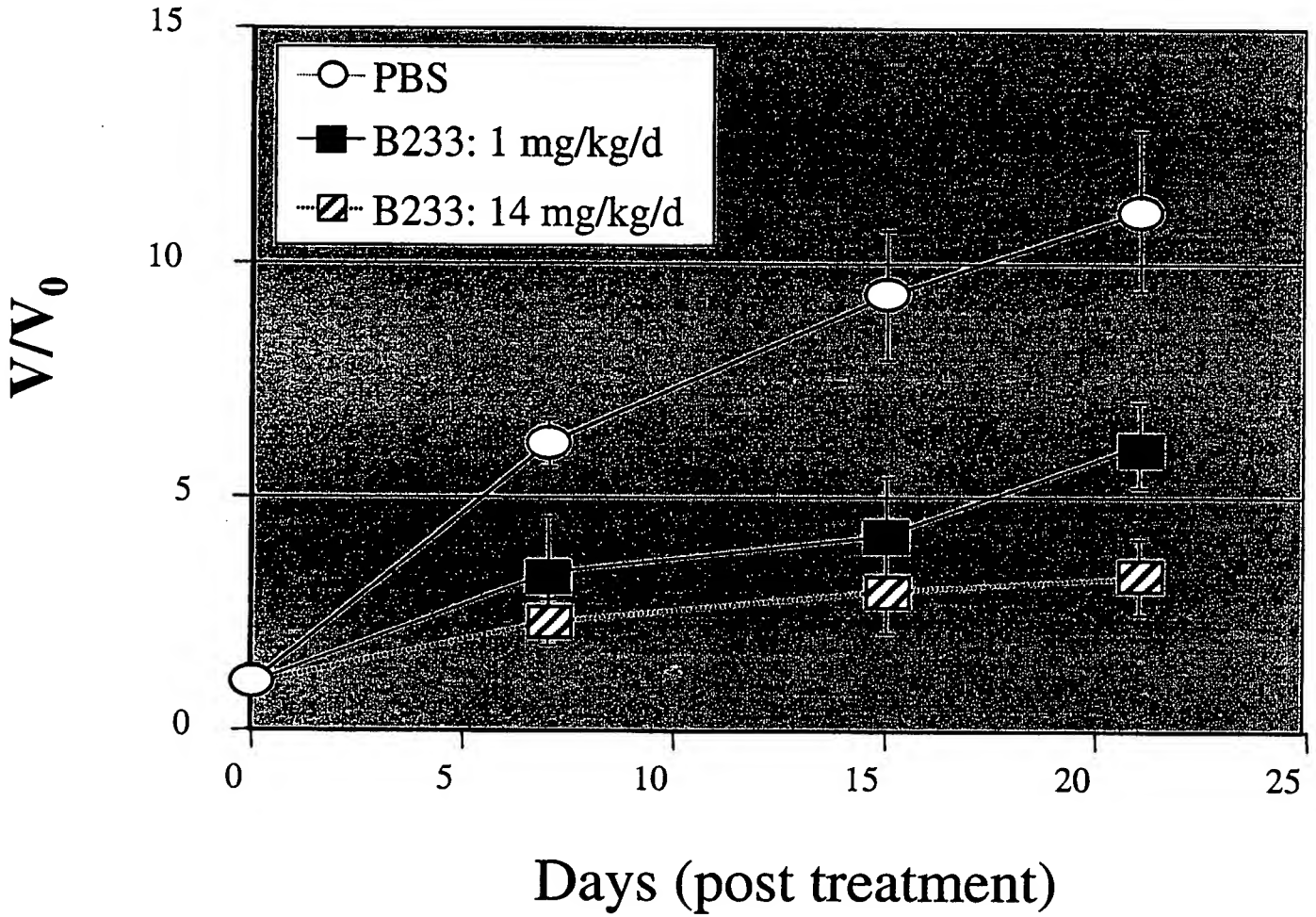


Exhibit E

A

MDA-MB-231

Control

Subject: C1 C2 C3 C4 C5 C6 C7 C8

B

EA2

Subject: E1 E2 E3 E4 E5 E6 E7 E8